March 10, 2011

Dr Warren Casey
Deputy Director
NICEATM
National Institute of Environmental Health Sciences
PO Box 12233, K2-16
Research Triangle Park, NC 27709

And via e-mail to: niceatm@niehs.nih.gov

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT ST.
NORFOLK, VA 23510
757-622-PETA
757-622-0457 (FAX)

Re: **76 FR 4113; January 24, 2011; Independent Scientific Peer Review Panel Meeting on an *In Vitro* Estrogen Receptor Transcriptional Activation Test Method for Endocrine Disruptor Chemical Screening; National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Request for Comments.**

Dear Dr Casey:

People for the Ethical Treatment of Animals (PETA) is the world's largest animal rights organization, with over 2 million members and supporters. We appreciate the opportunity to comment regarding the draft Background Review Document (BRD) and draft ICCVAM test method recommendations (TMR).

General Comments

We are pleased that ICCVAM is recommending the BG1Luc ER TA test method as a screening assay to identify substances with estrogen agonist and antagonist activity. We support the finding that this assay can be applied to a wide range of substances and can be routinely used to prioritize substances for further testing. We also appreciate the thoroughness of the BRD and the development of Performance Standards for the BG1Luc ER TA assay. We support the conclusion that the BG1Luc ER TA assay is equivalent to the OPPTS 890.1300/CERI STTA method; however, since the CERI STTA validation report has been published,[1] it would be useful to included a quantitative comparison and to compare chemicals used to assess accuracy as compared to ER binding and uterotrophic assays. While the BRD is thorough, it contains a large amount of repetitive information, which, if removed, could significantly shorten the document.

Additionally, we support any recommendations that could lead to reduction, refinement or replacement of animal testing. These include the recommendations that: 1) the BG1Luc ER TA

[1] OECD. 2006. Draft Report of Pre-validation and Inter-laboratory Validation For Stably Transfected Transcriptional Activation (TA) Assay to Detect Estrogenic Activity - The Human Estrogen Receptor Alpha Mediated Reporter Gene Assay Using hER-HeLa-9903 Cell Line. Available at: http://www.oecd.org/dataoecd/7/27/37504278.pdf (accessed 6 March 2011).

test be considered for quantitative, rather than just qualitative, assessment of estrogen agonist and antagonist activity,[2] 2) the BG1Luc ER TA test be incorporated as part of a weight of evidence approach to reduce or eliminate the need for testing in animal models such as the female rat pubertal, rat uterotrophic and fish short-term reproductive assays.[3]

While we are pleased with the results of the study, we have major concerns with the length of time it took to validate this test, which, when nominated in January 2004, already had a considerable amount of relevant data associated with it. We are disappointed that this review took seven years and was not completed in time for inclusion in the initial phase(s) of the Environment Protection Agency's Endocrine Disruptor Screening Program. The prolonged review has also affected development of a Performance Based Test Guideline (PBTG) for estrogen receptor transcriptional activation assays.

The delay of this validation process was likely exacerbated by the inclusion of 78 reference chemicals, many of which are not well characterized, in the validation process. New methods should be validated with reference chemicals whose activities are extremely well characterized. Following validation, other chemicals with suspected activity or limited data can then be characterized – it is inappropriate to mix the two. In addition, the ICCVAM list of 78 chemicals were described as chemicals "that should be used to standardize and validate *in vitro* ER and AR binding and TA test methods."[4] Since the BG1Luc test is concerned with ER TA agonist and antagonist effects only, we have to question why all 78 would be included.

Specific Comments

1.0 Draft ICCVAM Recommendations: the BG1Luc ER TA test method

Lines 36 – 37: The characterization of L-thyroxine as a "false negative" is misleading considering that this chemical is not well characterized (see Table 1). In fact, it later states in Section 5.2.1 that this substance was classified by ICCVAM as positive based on two reports of positive agonist activity and one report of no agonist activity, hardly a definitive set of evidence.

Lines 76 – 78: There seems to be something missing from this sentence.

Lines 99 – 110. Although assessment of both agonist and antagonist activity is an advantage of the BG1Luc ER TA test method over the CERI STTA method, transcriptional activation assays support but do not definitively prove receptor mediation. Binding studies are performed to confirm a receptor binding mechanism of action, and therefore cannot be replaced by a transcriptional activation assay. A more appropriate recommendation would be to validate an ER binding assay that uses a human recombinant ER. The CERI STTA method is currently being validated for antagonist activity.[5]

[2] NICEATM Draft ED BRD: BG1Luc ER TA Test Method – Section 5.0, p. 5-11.
[3] NICEATM Draft ED BRD: BG1Luc ER TA Test Method – Section 9.0, p. 9-2.
[4] NICEATM Draft ED BRD: BG1Luc ER TA Test Method – Section 1.0 Introduction, p. 1-3.
[5] Workplan for the Test Guidelines Programme. 2010. Organization for Economic Coordination and Development (OECD) (*www.**oecd**.org/data**oecd**/54/29/46034089.pdf*) (accessed 6 March 2010).

2.5.1 Solubility Testing

Solubility in 100% DMSO is not reflective of the solubility upon dilution in the culture medium – many compounds can be completely soluble in DMSO yet form precipitate when diluted in aqueous solution – this adjustment could lead to serious miscalculations of solubility in many cases.

3.0 Substances Used to Evaluate Test Method Accuracy

Table 3 – 2: The 78 reference substances, chosen based on "a preponderance of evidence found in a review of the scientific literature" includes several substances with very little information. The substances listed in Table 3-2 should be graded with respect to the confidence of a positive or negative determination based on the quantity and quality of available data as we have illustrated below in Table 1. In Table 1, substances with substantial, definitive data are not shaded, substances with a moderate amount of information are lightly shaded, and substances with little information are darkly shaded. Substances with low confidence (e.g. those darkly shaded) should be deleted from the reference list, and should not have been used in validation studies.

ICCVAM, in considering which substances to use to assess the accuracy of the agonist and antagonist activity, selected "only those substances that could be definitively classified as POS or NEG." Many (but not all) of the substances with little supporting data were not tested by ECVAM or Hiyoshi as indicated in **Table 2 for agonists and Table 3 for antagonists**.

Table 1. Copy of Table 3–2: Substances graded by amount of substantiating information.

ICCVAM Reference Substance	CASRN	ER TA Agonist Activity	ER TA Antagonist Activity	ER Binding Activity	CERI ER TA Activity	Uterotrophic Activity
12 – O – tetradecanoylphorbol-13-acetate	16561-29-8	PN (nt)	PN (nt)	PN (nt)	nt	nt
17-ß estradiol	50-28-2	POS (226/226)	PP (1/1)	POS (160/160)	POS	nt
17-α estradiol	57-91-0	POS (10/10)	PP (1/1)	POS (15/15)	POS	POS (nt/+)
17-α ethinyl estradiol	57-63-6	POS (21/21)	NEG (0/9)	POS (32/32)	POS	POS (+/+)
17ß-trenbolone	10161-33-8	PP (1/1)	PN (nt)	PN (nt)	POS	nt
19-nortestosterone	434-22-0	POS (3/3)	PP (1/1)	PP (1/7)	nt	nt
2-sec-butylphenol	89-72-5	PN (0/1)	PN (nt)	POS (2/2)	NEG	nt
2,4,5trichlorophenoxyacetic acid	93-76-5	PP (1/3)	PP (1/2)	PP (1/3)	nt	nt
4-androstenedione	63-05-8	PP (1/1)	PN (0/1)	PP (1/5)	NEG	nt
4-cumylphenol	599-64-4	POS (4/4)	PN (nt)	POS (3/3)	POS	nt
4-hydroxy androstenedione	566-48-3	PP (1/2)	PN (nt)	PP (nt)	nt	nt
4-hydroxytamoxifen	68047-06-3	PP (17/56)	POS (27/27)	POS (36/36)	nt	nt
4-tert-octylphenol	140-66-9	POS (20/23)	PN (nt)	POS (20/20)	POS	POS (nt/+)
5α-dihydrotestosterone	521-18-6	POS	NEG (0/3)	POS (17/18)	nt	POS (nt/+)

ICCVAM Reference Substance	CASRN	ER TA Agonist Activity	ER TA Antagonist Activity	ER Binding Activity	CERI ER TA Activity	Uterotrophic Activity
		(15/17)				
Actinomycin D	50-76-0	PN (nt)	PN (nt)	PN (nt)	nt	nt
Ammonium perchlorate	7790-98-9	PN (nt)	PN (nt)	PN (nt)	nt	nt
Apigenin	520-36-5	POS (25/25)	NEG (0/11)	POS	POS	nt
Apomorphine	58-00-4	PN (nt)	PN (nt)	PN (nt)	nt	nt
Atrazine	1912-24-9	NEG (0/29)	PN (0/1)	PP (2/19)	NEG	nt
Bicalutamide	90357-06-5	NEG (0/5)	PN (nt)	PN (nt)	nt	nt
Bisphenol A	80-05-7	POS (64/64)	NEG (0/12)	POS (46/47)	POS	POS (+/+)
Bisphenol B	77-40-7	POS (5/5)	PN (0/1)	POS (2/2)	POS	POS (nt/+)
Butylbenzyl phthalate	85-68-7	POS (11/13)	NEG (0/3)	POS (10/19)	POS	NEG (-/-)
Chrysin	480-40-0	POS (6/9)	NEG (0/4)	PP (2/10)	nt	nt
Clomiphene citrate	50-41-9	POS (3/4)	PP (1/1)	POS (8/8)	POS	nt
Corticosterone	50-22-6	NEG (0/5)	PP (1/3)	NEG (0/6)	NEG	nt
Coumestrol	479-13-0	POS (29/29)	NEG (0/8)	POS (38/38)	POS	nt
Cycloheximide	66-81-9	PN (nt)	PP (nt)	PN (nt)	nt	nt
Cyproterone acetate	427-51-0	PP (1/6)	PN (0/1)	PP (1/2)	nt	nt
Daidzein	486-66-8	POS (38/38)	NEG (0/6)	POS (32/35)	POS	POS (nt/+)
Dexamethasone	50-02-2	PP (2/6)	PP (1/1)	PP (1/4)	nt	nt
Di-n-butyl phthalate	84-74-2	PP (5/10)	NEG (0/3)	POS (7/13)	nt	NEG (-/-)
Dibenzo[a.h] anthracene	53-70-3	PP (1/2)	PP (nt)	PN (0/1)	nt	nt
Dicofol	115-32-2	POS (4/6)	NEG (0/2)	POS (2/2)	nt	nt
Diethylhexyl phthalate	117-81-7	PP (4/9)	NEG (0/3)	PP (4/8)	NEG	NEG (nt/-)
Diethylstilbestrol	56-53-1	POS (41/41)	NEG (0/2)	POS (52/52)	POS	nt
Estrone	53-16-7	POS (25/27)	PP (1/2)	POS (29/29)	POS	POS (nt/+)
Ethyl paraben	120-47-8	POS (5/5)	PN (nt)	POS (4/5)	POS	nt
Fenarimol	60168-88-9	POS (5/6)	PN (0/1)	POS (2/2)	nt	nt
Finasteride	98319-26-7	PN (nt)	PN (0/1)	PN (0/1)	nt	nt
Flavone	525-82-6	PP (2/5)	PP (1/1)	PP (3/13)	nt	nt
Fluoranthene	206-44-0	PN (nt)	PN (nt)	PN (0/1)	nt	nt
Fluoxymestrone	76-43-7	PN (nt)	PN (nt)	PN (0/1)	nt	nt
Flutamide	13311-84-7	NEG (0/5)	PN (0/1)	NEG (0/2)	nt	nt
Genistein	446-72-0	POS (99/101)	NEG (0/13)	POS (64/64)	POS	POS (+/+)
Haloperidol	52-86-8	PN (0/1)	PN (nt)	PN (0/1)	nt	nt
Hydroxyflutamide	52806-53-8	NEG (0/2)	PN (nt)	PP (1/4)	nt	nt
Kaempferol	520-18-3	POS (22/22)	NEG (0/9)	POS (19/19)	POS	nt
Kepone	143-50-0	POS (13/17)	NEG (0/2)	POS (14/15)	POS	nt
Ketoconazole	65277-42-1	PN (0/1)	PN (nt)	PN (0/1)	NEG	nt
L-thyroxine	51-48-9	POS (2/3)	PN (nt)	POS (2/2)	nt	nt
Linuron	330-55-2	NEG (0/7)	PN (nt)	POS (2/3)	NEG	nt

ICCVAM Reference Substance	CASRN	ER TA Agonist Activity	ER TA Antagonist Activity	ER Binding Activity	CERI ER TA Activity	Uterotrophic Activity
Medroxyprogesterone acetate	71-58-9	PP (1/2)	PN (0/1)	POS (2/2)	NEG	nt
meso-hexestrol	84-16-2	POS (3/3)	PN (nt)	POS (11/11)	nt	nt
Methyl testosterone	58-18-4	POS (4/5)	PP (1/2)	POS (2/3)	POS	nt
Mifepristone	84371-65-3	PP (3/6)	NEG (0/3)	POS (4/6)	NEG	nt
Morin	480-16-0	PP (1/1)	PN (nt)	POS (3/3)	POS	nt
Nilutamide	63612-50-0	PN (nt)	PN (nt)	PN (nt)	nt	nt
Norethynodrel	68-23-5	POS (4/4)	NEG (2/2)	POS (7/7)	POS	na
o.p'-DDT	789-02-6	POS (24/25)	NEG (0/3)	POS (20/22)	nt	POS (+/nt)
Oxazepam	604-75-1	PN (nt)	PN (nt)	PN (nt)	nt	nt
p-n-nonylphenol	104-40-5	POS (9/9)	NEG (0/2)	POS (21/21)	NEG	IC (+/-)
p.p'-DDE	72-55-9	POS (5/7)	NEG (2/2)	PP (5/15)	nt	nt
p.p'-methoxychlor	72-43-5	POS (23/26)	PP (1/5)	POS (16/26)	POS	IC (+/-)
Phenobarbital	50-06-6	NEG (0/2)	PN (nt)	PN (0/1)	nt	nt
Phenolphthalin	81-90-3	PN (0/1)	PN (nt)	POS (2/2)	NEG	nt
Pimozide	2062-78-4	PN (nt)	PN (nt)	PN (nt)	nt	nt
Procymidone	32809-16-8	NEG (0/4)	PN (nt)	PP (2/5)	nt	nt
Progesterone	57-83-0	PP (3/15)	NEG (0/2)	PP (2/20)	NEG	nt
Propylthiouracil	51-52-5	PN (nt)	PN (nt)	PN (nt)	nt	nt
Raloxifene HCl	82640-04-8	PP (7/31)	POS (13/13)	POS (16/16)	NEG	nt
Reserpine	50-55-5	PN (0/1)	PN (nt)	PN (0/1)	NEG	nt
Resveratrol	501-36-0	POS (24/37)	NEG (0/16)	POS (9/12)	nt	nt
Sodium azide	26628-22-8	PN (0/1)	PN (nt)	PN (nt)	nt	nt
Spironolactone	52-01-7	NEG (0/3)	PN (nt)	PN (0/1)	NEG	nt
Tamoxifen	10540-29-1	POS (15/22)	POS (20/22)	POS (46/46)	POS	nt
Testosterone	58-22-0	PP (4/9)	PN (0/1)	PP (5/12)	POS	nt
Vinclozolin	50471-44-8	PP (6/13)	PN (0/1)	POS (3/5)	POS	nt

4.2.9 Weak Agonist Positive Control: Flavone

It is not clear why flavone was chosen as the weak antagonist positive control as there is scant data to support such a conclusion. The extremely high CV's noted indicate that estrogen antagonist activity of flavones is variable and is a poor candidate for a control substance.

4.3 Solubility Test Results

It does not appear that differences among the labs in range finder starting concentrations were ever fully explained. Initially in Phases 1 and 2, this was attributed to problems associated with log scale dilutions in the 1% DMSO medium. Protocols were modified after Phase 2 to use test substance solubility in 100% DMSO as the starting concentration for range finder testing. However, differences persisted in Phase 3 (Tables 4-11 and 4-12) and all three labs rarely had the same starting concentration for each substance tested.

4.4.2 BG1Luc ER TA Agonist and Antagonist Data

The table numbers in lines 250-251 should be 4-12, 4-14 and 4-15, not 4-11, 4-12, and 4-13.

5.0 Accuracy of the BG1Luc ER TA

5.1 Substances Used for Accuracy Analysis

Table 5-2: Most of the substances with little supporting data were not tested by either ECVAM or Hiyoshi (Tables 2 and 3 below) and it is not clear why they are included in the analysis. If a substance is not tested in two out of three laboratories during the validation, a consensus determination cannot be established.

The discordance in lab results for atrazine, corticosterone, and dicofol (Table 5-2) was never fully explained in the report. Atrazine and corticosterone are well-substantiated negative agonists, yet ECVAM reported a positive response for these. The discordance with dicofol (two positives, one negative) may be illustrative of the moderate amount of substantiating evidence for this substance.

Table 2. Copy of Table 5-2: Agonist substances with little or moderate substantiating data indicated.

Agonist		Classification							
Substance	**CASRN**	**ICCVAM**	**Lumi Cell**	**XDS**		**ECVAM**		**Hiyoshi**	
17α-Estradiol	57-91-0	POS	POS	POS	(1/1)	POS	(3/3)	POS	(2/2)
17α-Ethinyl Estradiol	57-63-6	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)
17ß-Estradiol	50-28-2	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
19-Nortestosterone	434-22-0	POS	POS	POS	(1/1)	NT		NT	
4-Cumylphenol	599-64-4	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
4-tert-Octylphenol	140-66-9	POS	POS	I	(1/1)	POS	(1/1)	POS	(2/2)
5α-Dihydrotestosterone	521-18-6	POS	I	I	(1/1)	I	(1/1)	POS	(1/1)
Apigenin	520-36-5	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
Atrazine	1912-24-9	NEG	NEG	NEG	(3/3)	POS	(3/3)	NEG	(3/3)
Bicalutamide	90357-06-5	NEG	NEG	NEG	(1/1)	NT		NT	
Bisphenol A	80-05-7	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)
Bisphenol B	77-40-7	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)
Butylbenzyl phthalate	85-68-7	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)
Chrysin	480-40-0	POS	POS	POS	(2/2)	NT		NT	
Clomiphene citrate	50-41-9	POS	I	I	(1/1)	NEG	(1/1)	POS	(1/1)
Corticosterone	50-22-6	NEG	NEG	NEG	(3/3)	POS	(3/3)	NEG	(3/3)
Coumestrol	479-13-0	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
Daidzein	486-66-8	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
Dicofol	115-32-2	POS	POS	POS	(1/1)	NEG	(1/1)	POS	(1/1)
Diethylstilbestrol	56-53-1	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)
Estrone	53-16-7	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)

Agonist		Classification							
Substance	**CASRN**	**ICCVAM**	**Lumi Cell**	**XDS**		**ECVAM**		**Hiyoshi**	
Ethyl paraben	120-47-8	POS	POS	I	-1	POS	(1/1)	POS	(1/1)
Fenarimol	60168-88-9	POS	POS	POS	(1/1)	NT		NT	
Flutamide	13311-84-7	NEG	I	I	-1	NT		NT	
Genistein	446-72-0	POS	POS	POS	(3/3)	POS	(3/3)	POS	(4/4)
Hydroxy Flutamide	52806-53-8	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Kaempferol	520-18-3	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
Kepone	143-50-0	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
L-Thyroxine	51-48-9	POS	NEG	NEG	(1/1)	NT		NT	
Linuron	330-55-2	NEG	NEG	NEG	(1/1)	NT		NT	
meso-Hexestrol	84-16-2	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
Methyl testosterone	58-18-4	POS	POS	POS	(3/3)	POS	(1/1)	POS	(2/2)
Norethynodrel	68-23-5	POS	POS	POS	(2/2)	POS	(1/1)	POS	(2/2)
o,p'-DDT	789-02-6	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)
p-n-Nonylphenol	104-40-5	POS	POS	POS	(3/3)	POS	(3/3)	POS	(2/3)
p,p'- Methoxychlor	72-43-5	POS	POS	POS	(1/1)	POS	(1/1)	POS	(2/2)
p,p'-DDE	72-55-9	POS	I	I	(1/1)	I	(1/1)	NEG	(1/1)
Phenobarbital	50-06-6	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NT	
Procymidone	32809-16-8	NEG	I	I	(1/1)	NT		NT	
Resveratrol	501-36-0	POS	I	POS	(1/1)	I	(1/1)	NEG	(1/3)
Spironolactone	52-01-7	NEG	NEG	NEG	(1/1)	NT		NT	
Tamoxifen	10540-29-1	POS	I	I	(1/1)	I	(1/1)	POS	(1/1)

Table 3. Copy of Table 5-3: Antagonist substances with little or moderate substantiating data indicated.

Antagonist		Classification							
Substance	**CASRN**	**ICCVAM**	**Lumi Cell**	**XDS**		**ECVAM**		**Hiyoshi**	
17α-Estradiol	57-91-0	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
4-Hydroxytamoxifen	68047-06-3	POS	POS	POS	(1/1)	I	(2/2)	POS	(1/1)
5α-Dihydrotestosterone	521-18-6	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Apigenin	520-36-5	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(4/4)
Bisphenol A	80-05-7	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Butylbenzyl phthalate	85-68-7	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(4/4)
Chrysin	480-40-0	NEG	NEG	NEG	(1/1)	NT		NT	
Coumestrol	479-13-0	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Daidzein	486-66-8	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Di-n-butyl phthalate	84-74-2	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Dicofol	115-32-2	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Diethylhexyl	117-81-7	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)

phthalate									
Diethylstilbestrol	56-53-1	NEG	NEG	NEG	(1/1)	NEG	(1/1)	POS	(1/1)
Genistein	446-72-0	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(3/3)
Kaempferol	520-18-3	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Kepone	143-50-0	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Mifepristone	84371-65-3	NEG	NEG	NEG	(1/1)	NT		NT	
Norethynodrel	68-23-5	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
o,p'-DDT	789-02-6	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(3/3)
p-n-Nonylphenol	104-40-5	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(3/3)
p,p'-DDE	72-55-9	NEG	NEG	NEG	(1/1)	NEG	(1/1)	NEG	(1/1)
Progesterone	57-83-0	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(3/3)
Raloxifene HCl	82640-04-8	POS	POS	POS	(1/1)	POS	(1/1)	POS	(1/1)
Resveratrol	501-36-0	NEG	NEG	NEG	(3/3)	NEG	(3/3)	NEG	(3/3)
Tamoxifen	10540-29-1	POS	POS	POS	(3/3)	POS	(3/3)	POS	(3/3)

5.4 Comparison of BG1Luc ER TA Results with CERI STTA (OPPTS 890.1300)

This qualitative comparison is helpful for determining the relative utility of the two assays; however, it would be more informative to include a quantitative comparison as well, as we have done in **Table 4** below. During the OECD validation of the CERI STTA assay, it was decided that a useful exercise would be to use the ER STTA assay as a proof-of-concept for a Performance-Based Test Guideline (PBTG). The objective is to use two validated assays, in this case the CERI STTA assay and now the BG1Luc ER TA assay (agonist version) to create a set of performance standards that can be used to evaluate and expedite validation of subsequent similar assays. To compare assays that may generate different types of data and utilize different decision criteria, it is useful to present data as a Relative Potency Index (RPI) in addition to EC_{50}. The RPI is the EC_{50} of the positive control divided by the EC_{50} of the chemical multiplied by 100. We suggest that the RPI be added to Table 5-7.

In addition, several chemicals that were tested in the validation of the CERI STTA method are missing from the comparison in Table 5-7, including clomiphene citrate, methoxychlor and tamoxiphen.

6.0 Test Method Reliability

6.1.6 Antagonist E2 Control Values

Line 185: Table 6-3 should be Table 6-6.

9.0 Animal Welfare Considerations

Lines 32 – 35: Contain a direct repeat of lines 17 – 19 and should be deleted.

Based on a 97% concordance (33/34) of findings from the BG1Luc ER TA assay and the ER rat cytosol binding assay it is suggested that the former could serve as a replacement for the latter. Following the same logic, a 92% concordance (12/13, with no false negatives) should argue for

that the BG1Luc ER TA assay could replace the uterotrophic assay. In fact, in the interest of reducing animal use, a strong recommendation should be made to investigate the use of *in vitro* metabolizing systems with the BG1Luc ER TA assay so that the ER TA could definitively replace the uterotrophic assay.

Lines 52 – 54: Contain a direct repeat of lines 20 -22 and should be deleted.

10.1.3 BG1LUC 4E2 Cell line

If the line is available only from a private academic lab, will supply and quality control (e.g. passage number) be an issue?

10.3 Time and Cost Considerations

Lines 67 and 75: Authors' names are misspelled: should be Willett and Sullivan.

In conclusion, we find the BG1Luc test to be an accurate method for both qualitatively and quantitatively assessing the ER TA agonist and antagonist potential of a wide range of substances. We urge you to further enhance the utility of this method by pursuing many of the recommendations in the report as well as our recommendations, such as incorporating the use of *in vitro* metabolizing systems. We also ask that you reconsider your list of 78 substances when validating future ER/AR binding and TA tests and only use chemicals that have been definitively evaluated for their effects. Finally, in light of the need for new tests that can reduce, refine or replace animals in testing, we suggest a thorough examination of the validation process used in this study to determine if there are ways to make future studies more streamlined and time-efficient while still meeting the needs of public health and welfare.

Sincerely,

/s/

Catherine Willett, PhD
Science Policy Advisor
Regulatory Testing Division
Tel: 617-522-3487

/s/

Patricia L. Bishop, M.S.
Research Associate
Regulatory Testing Division
Tel: 757-390-0564